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TRP Channels in Pain and Inflammation: Therapeutic Opportunities

Mark A. Schumacher, Ph.D., M.D.

Abstract

In ancient times, physicians had a limited number of therapies to provide pain relief. Not surprisingly, plant extracts applied topically often served as the primary analgesic plan. With the discovery of the capsaicin receptor (TRPV1), the search for 'new' analgesics has returned to compounds used by physicians thousands of years ago. One such compound, capsaicin, couples the paradoxical action of nociceptor activation (burning pain) with subsequent analgesia following repeat or high-dose application. Investigating this 'paradoxical' action of capsaicin has revealed several overlapping and complementary mechanisms to achieve analgesia including receptor desensitization, nociceptor dysfunction, neuropeptide depletion and nerve terminal destruction. Moreover, the realization that TRPV1 is both sensitized and activated by endogenous products of inflammation including bradykinin, H+, ATP, fatty acid derivatives, NGF and trypsins, has renewed interest in TRPV1 as an important site of analgesia. Building on this foundation, a new series of preclinical and clinical studies targeting TRPV1 have been reported. These include trials using brief exposure to high-dose topical capsaicin in conjunction with prior application of a local anesthetic. Clinical use of resiniferatoxin (RTX), another ancient but potent TRPV1 agonist, is also being explored as a therapy for refractory pain. The development of orally-administered high affinity TRPV1 antagonists hold promise for pioneering a new generation of analgesics capable of blocking painful sensations at the site of inflammation and tissue injury. With the isolation of other members of the TRP channel family such as TRPA1, additional opportunities are emerging in the development of safe and effective analgesics.

Keywords

Analgesics; Non-Narcotic; Pain; Sensory Receptors; transient receptor potential cation channel; TRP channels; TRPV1; capsaicin receptor

Introduction

Since ancient times, physicians have faced a common dilemma: How does one effectively relieve a patient's pain? Long before the advent of randomized double-blinded clinical trials, early physicians successfully used native plant derivatives to provide pain relief. Although their preparations may have been crude by today's standards, they set in motion a path of discovery that has resulted in the current revolution in novel analgesic development. Now there is evidence that compounds used by ancient physicians to treat painful conditions from arthritis to toothaches contain unique chemicals that can block a peripheral sensory neuron's ability to detect painful stimuli. Moreover, the molecular identity of these compounds is helping to reveal how noxious thermal, mechanical and chemical stimuli are detected and

Correspondence: Mark A. Schumacher Ph.D., M.D., Department of Anesthesia and Perioperative Care, University of California, San Francisco, 513 Parnassus Ave Rm. S436 (Box S436), San Francisco, CA 94143-0427, (o) 415-502-7022, (f) 415-514-0185, schumacm@anesthesia.ucsf.edu.

signal persistent states of tissue injury and inflammation. As discussed later, the capsaicin receptor, also known as TRPV1, is an archetype for a broader family of ion channels that likely transduce virtually all modalities of painful stimuli. As such, progress has been made both at the bench and bedside to develop therapeutics that selectively target the TRP-family of pain-transducing channels providing an exquisite alternative to opioid-based analgesics.

What are the features of an ideal analgesic? A clinician's viewpoint likely includes: acts selectively on the "pain-sensing" nerves, does not depress the central nervous system or respiration, maintains an analgesic effect over time, is easy to administer, is not addictive and is inexpensive. Will such a compound ever be found or synthesized? Perhaps, the future of the 'ideal' analgesic has been with us all along.

Ancient analgesics

Some of the earliest written accounts in Western civilization describing analgesic compounds, especially topical agents, date back to Roman times more than 2000 years ago (50 BC – 23 AD). Although popular legend contends that the Roman physician, Euphorbius, first used the resin from *Euphorbia resinifera* to treat the arthritic pain suffered by Emperor Augustus, it was actually King Juba II of Mauretania, that scholars believe is responsible for its discovery [1]. *E. resinifera* is a cactus like plant indigenous to the Anti-Atlas Mountains of North Africa (Morocco). This spurge contains a toxic latex that when dried, resolves into a potent resin capable of inducing topical analgesia and reducing the pain of a toothache. Surprisingly, its medicinal use continued for hundreds of years before largely vanishing in the 1700's. Predating the accounts of *E. resinifera* during Roman times was the medicinal use of hot chilies in South America dating as far back as 4000 BC. However, much of our modern accounts and written records of chili's irritant properties and medicinal use are derived from Aztec culture beginning in the 12th century. Aztec's were a highly disciplined culture where a child's inappropriate behavior could result in being held over a pile of burning chili peppers! Fortunately, Aztec physicians of that time also realized chili's usefulness to treat painful maladies. Nevertheless, it was not until Columbus returned to Europe with chili 'peppers' in the late 1400s that its culinary and medicinal attributes began to spread throughout the modern world.

Where does pain start?

In the peripheral nervous system, somatosensory detection of tissue damaging stimuli occurs at the peripheral terminals of primary afferent neurons whose cell bodies reside in the trigeminal and dorsal root ganglia. These specialized nociceptive neurons innervate essentially all tissues within the body with the exception of the brain parenchyma. Those neurons that respond to tissue damage (intense mechanical, thermal or noxious chemical stimuli) express specialized proteins in their nerve terminals capable of pain transduction and have been termed primary afferent nociceptors or 'nociceptors'. Nociceptors have many distinguishing features when compared with other peripheral neurons. Importantly, they are relatively small in size, and are either unmyelinated (C-type) or thinly myelinated (A delta-type) explaining their relatively slow conduction velocities. Under conditions of noxious stimuli, the nociceptor terminals detect impending or actual tissue injury and elicit a complex barrage of electrochemical activity which subsequently signals second order neurons in lamina I, II and V of the dorsal horn of the spinal cord. Ultimately, nociceptive signaling is transmitted to higher centers of the central nervous where it is perceived as a harmful or unpleasant experience [2].

Within the trigeminal (V) and dorsal root ganglia (DRG) reside the cell bodies for the majority of nociceptive fibers that populate cranial nerves V (innervation of the majority of the face, conjunctiva, mouth and dura mater) as well as cranial nerves VII, IX and X

(innervation of the skin of the external ear, and mucous membranes of the larynx and pharynx) [3]. Likewise, nociceptor terminals derived from the spinal dorsal root ganglia (cervical, thoracic, and lumbar) innervate the somatotopic dermatomes of the skin and underlying tissue and visceral organs. Vagal afferents provide a second source of visceral innervation from cell bodies located within the nodose (inferior vagal) ganglion. Despite dual sensory innervation of the majority of internal organs, afferents involved with the transduction of painful visceral stimuli (ischemia, stretch, distension) are primarily derived from the dorsal root ganglia [4]. Nevertheless, vagal afferents still play a role in pain transduction even though their function may be more 'global' in nature, providing feedback loops to the brain and neuroendocrine systems resulting in systemic pain modulation and associated perceptions of nausea, malaise or impending doom [5].

Nociceptors

Nociceptors are also characterized based on their threshold for evoking a sensation of pain including noxious chemical, thermal (temperatures ≥ 43–45 °C) or mechanical stimuli. Recent work has focused on further subdividing nociceptive neurons based on their adult expression of associate neuropeptides and receptor proteins. Specific antibody staining is now available for the detection of subtypes of epidermal nociceptive fibers in human volunteers. In general, this has revealed at least two additional subcategories of small-diameter nociceptive neurons: Peptidergic, containing substance P and CGRP, with co-expression of the nerve growth factor (NGF) receptors TrkA and p75, or Non-peptidergic sensory neurons that lack neuropeptides and TrkA receptors but express the antigen Isolectin - B4 [6–8]. Although both subtypes initially required NGF during development, their adult phenotypes differ based on the down regulation and loss of TrkA receptors on the nonpetidergic nociceptors. Despite this elegant classification of nociceptor subtypes, discharge patterns of polymodal nociceptors do not correlate with stimulus-induced pain sensation [7]. Therefore, central processing of nociceptor impulses must be required for the discrimination of painful sensations.

As neuroscientists investigated the characteristics of nociceptor physiology in cultured sensory neurons, inward current responses to noxious heat (I_{heat}) [9–12], mechanical stimuli (I_{mech}) [13] and chemical stimuli were observed in small-diameter sensory neurons [14]. In particular, a subset of sensory neurons were activated by capsaicin, the principle pungent component in hot chili peppers. Structurally, capsaicin contains a homovanillic acid group that is important for its pungent activity. Therefore capsaicin and its related derivatives are usually referred to as 'vanilloid compounds'. In mammals, exposure to capsaicin produces excitation of nociceptors with secondary release of inflammatory and vasoactive peptides [15]. In humans, intradermal injection of capsaicin produces immediate burning pain, similar to that reported with noxious thermal stimuli or in certain painful neuropathies [2].

The Capsaicin Receptor (TRPV1)

Prior to the isolation of a cDNA encoding a functional capsaicin (vanilloid) receptor, evidence that the effects of capsaicin may be mediated by a receptor began with measuring the dose-dependent effects of capsaicin and its analogues on protective eye wiping behavior in the rat. Subsequently, dose response experiments measuring calcium influx and current responses in cultured sensory neurons were completed [16]. In addition, resiniferatoxin (RTX), a diterpine derived from the latex of the plant *Euphorbia resinifera* used by the ancient physicians of Roman times, was found to share structural similarity to capsaicin by containing a common vanilloid moiety essential for activity (Figure 1). Both capsaicin and RTX induce a dose-dependent influx of calcium in cultured sensory neurons. RTX has an apparent nanomolar binding affinity in DRG membranes and was originally utilized as a

high affinity radioligand for the characterization of purported vanilloid binding sites [17]. Several comprehensive reviews have been published encapsulating early work on vanilloid receptor biology [17, 18].

TRPV1 is an ion channel that integrates multiple noxious stimuli in nociceptors

With the isolation of a cDNA clone encoding a capsaicin-activated ion channel in 1997, the molecular basis of the vanilloid receptor -VR1 (TRPV1) was finally realized [19]. Now termed TRPV1 (transient receptor potential cation channel, subfamily V, member 1), it encodes a nonselective cation channel subunit of approximately 95kDa that is highly expressed in the small-diameter sensory neurons of dorsal root, trigeminal and vagal ganglion. Its structure (Figure 2) most resembles that of members of the K_v 1.2 and store operated channel family [19]. The TRPV1 subunit spans the plasma membrane six times containing large N- and C-terminal intracellular regions and is proposed to form tetrameric and/or heteromeric channel complexes [20–22]. It is activated by capsaicin and RTX on the intracellular surface in a dose-dependent manner. Once activated, TRPV1 is not selective for monovalent cations; rather it preferentially conducts calcium through its channel pore resulting in an increase in intracellular calcium and cellular depolarization.

The role of TRPV1 on Inflammatory Pain and Hyperalgesia

The pain and disability from chronic inflammatory conditions remains widespread and difficult to manage despite a variety of available pharmacologic therapies. With the realization that TRPV1 could be activated by thermal stimuli, it was initially considered to serve a restricted role for nociceptive transduction - the acute detection of heat in the noxious range. Importantly, TRPV1 was not found to be simply a thermal detector but also a critical part of a system designed to signal potential and/or ongoing pathophysiological conditions that if left uncorrected, could lead to irreversible cellular injury.

Nociceptors have the ability to adjust their sensitivity following repetitive noxious stimuli or tissue injury. *Sensitization* encompasses an increase in spontaneous nociceptor activity, a lowered threshold for activation, and an increase in action potential firing after suprathreshold stimuli [2]. Under these circumstances, prolonged nociceptor activation may be warranted to ensure protective behavioral responses. Together with plasticity changes in the dorsal horn of the spinal cord, local nociceptor sensitization contributes an essential role in the initiation and maintenance of hyperalgesia. A turning point in the realization that TRPV1 was critical to the signaling of inflammatory pain and hyperalgesia was the finding that TRPV1 −/− null mice failed to develop thermal hyperalgesia following exposure to peripheral inflammation [23, 24]. Therefore, TRPV1 serves as a critical molecular site of nociceptor sensitization where the action of both an ***inflammatory mediator*** and a ***noxious stimulus (heat)*** are required for nociceptor activation.

Endogenous Activation and Sensitization of TRPV1

Nociceptive ion channels and, by definition, the nociceptors expressing them, may serve more to signal ongoing tissue injury and inflammation rather than acute noxious stimuli. This suggests a sensory system that can distinguish between an acute noxious stimulus and a chronic painful condition such as inflammation. It also suggests that compounds capable of selectively blocking the activation of this class of receptor/channels could theoretically spare normal sensations of touch or extremes of heat. Although the identity of endogenous agents capable of activating TRPV1 continues to emerge, a number of these have been shown to either act directly or sensitize TRPV1 through secondary messengers and/or protein modification. As summarized (Table 1), multiple agents and pathways are acting in concert to modulate TRPV1 under conditions of tissue injury/inflammation and nerve injury.

Protons

Protons (H^+) as found in excess under acidic conditions (low pH) have been shown to potentiate both the vanilloid and noxious thermal response of TRPV1 through direct activation of TRPV1 *in vitro* [19, 32]. Moreover, an extracellular site essential for proton-induced activation of TRPV1 has been identified and apparently differs from the sites that mediate capsaicin and heat activation [35, 51]. Although the detection of 'mild' acidic conditions, pH 7.0–7.4, may be mediated by a family of acid-sensing sodium channels [52], the ability of hydrogen ions to potentiate or directly activate TRPV1 suggests a nociceptive role under pathophysiologic conditions of ischemia or infection. Additional behavioral studies should help determine the degree to which TRPV1 participates *in vivo* to proton-mediated nociception [23, 24].

Bradykinin

(BK) a naturally occuring inflammatory nonapeptide, has been shown to directly activate nociceptors as well as to produce nociceptor sensitization through several mechanisms [53]. The effects of bradykinin are mediated through two receptor subtypes: B_1 and B_2. The B_2 receptor is widely expressed being responsible for the majority of BK-induced effects including those in nociceptors. In contrast, the B_1 subtype is expressed in lower abundance and has a higher affinity for the BK metabolite, Des [Arg^9] bradykinin. B_1 receptors are upregulated under conditions of injury/inflammation and have been shown to contribute to inflammatory hyperalgesia [54]. Activation of PKC produces C-fiber type nociceptor depolarization and activated forms of PKC are associated with the phosphorylation of selective domains of receptors and ion channels [55]. A PKC isozyme (epsilon), PKCe, has been implicated in nociceptor function as it may mediate a component of NGF-mediated hyperalgesia [56]. Furthermore, PKCe mutant mice have reduced mechanical and thermal hyperalgesia but have normal baseline thresholds for noxious stimuli [56]. Nevertheless, BK activation of nociceptors was eliminated in B_2 deficient mice, reaffirming the role of the B_2 receptor as the predominant target for BK action on nociceptors [57]. Moreover, at least two potential pathways have been described that could link BK to TRPV1 activation in nociceptors: 1) BK activation of phospolipase A2 with subsequent metabolism of arachidonic acid into products of the lipoxygenase pathway [41], and 2) BK-mediated production of diacylglycerol and inositol 1,4,5 trisphosphate with subsequent activation of PKC [58].

ATP

The hypothesis that adenosine triphosphate (ATP) plays an important role in the synaptic transmission of sensory neurons began with the early observations of Holton and Holton [59]. Cellular activation in response to ATP revealed that one or more 'fast' ATP gated channels may exist in various tissues, including nociceptors. Furthermore, it is plausible that ATP released from injured cells functions as a signal of tissue injury. Of importance is the ATP gated channel subtype $P2X_3$, which is predominantly expressed in small-diameter sensory neurons and is proposed to be one mechanism that mediates ATP-induced activation of nociceptors [60]. $P2X_3$ may play a role in enhancing thermal and/ormechanical transduction under inflammatory or pathophysiologic conditions [61]. More recently, the metabotropic G-protein coupled receptor, $P2Y_2$, has been shown to have a direct link to TRPV1 activation and sensitization [44–46].

Lipids: Fatty acid metabolites

Nociceptors are sensitized by a wide range of inflammatory products of arachidonic acid (AA) metabolism. These include certain products of the cyclooxygenase pathway (PGE_2, PGI_2) that are known to exert their biological action through G-protein coupled receptors

and more recently isoprostanes, compounds that are formed by nonenzymatic peroxidation of AA such as 8-iso PGE_2 and 8-iso $PGF2_a$. Alternately, AA is metabolized via the lipoxygenase pathway, producing a multitude of products including LTB4, and 15-S-di HETE that have been shown to sensitize nociceptors. Although lipoxygenase products have a wide range of biological activities and their receptor targets were previously minimally characterized, 12-S-HPETE and LTB4 have been recently shown to directly activate TRPV1 [41]. More recently, two derivatives of dopamine (N-arachidonoyl-dopamine (NADA) and N-oleoyl-dopamine) have also been found to activate TRPV1 and are associated with experimental hyperalgesia [62, 63].

Nerve Growth Factor (NGF)

Since its identification by Levi-Montalcini and Calissano, NGF has been distinguished from other neurotrophin family members (BDNF, NT-3 and NT-4/5) as being essential for normal nociceptor development and function [64]. NGF is synthesized and secreted by a wide variety of tissues including Schwann cells located within sensory ganglion and importantly in the end-target tissues of nociceptive terminals - epidermal fibrobroblasts and keratinocytes. NGF is intimately involved in maintaining and modifying the phenotype of the nociceptor population. Adult sensory neurons lose their dependency on NGF for survival but retain expression of its high affinity receptor-TrkA, primarily on the small-diameter primary afferent nociceptors (C and A-delta) [65]. Conditions of inflammation that are characterized by inflammatory cell migration, cytokine release, edema, erythema, pain and hyperalgesia, can be experimentally modeled by injection of Complete Freund's Adjuvant (CFA) into the hind paw of the rat. Following CFA injection, one finds increased NGF production and content at the site of injury serving as the driving signal for the associated pain and hyperalgesia [66–68].

Use of IgG-Trk fusion protein and anti-NGF antibodies have been shown to block inflammatory models of pain and thermal hyperalgesia despite continued evidence of erythema and edema [65]. Human studies also corroborate a role for NGF in peripheral pain transduction. Intradermal injection of NGF in human volunteers induces thermal hyperalgesia and mechanical allodynia at the site of injection, beginning as early as three hours and lasting up to 21 days (66). NGF is also detected in the synovial fluid of patients with rheumatic disease or other types of chronic arthritis [69]. Patients with congenital insensitivity to pain with anhydrosis (CIPA) have an absence of reaction to noxious stimuli and have been shown to contain mutations within the gene that encodes the NGF – TrkA receptor [70].

A major consequence of NGF production in peripheral inflammation is TRPV1 -mediated pain and thermal hyperalgesia. It is now emerging that NGF has at least three principle actions on TRPV1 in nociceptors: 1) Modification of the TRPV1 channel structure changing its sensitivity towards activation - lowering its threshold of thermal activation [47], 2) Increasing the transport of the TRPV1 channel protein to the plasma membrane, thereby making more TRPV1 receptor immediately available for a greater cellular response under activating conditions [48], 3) Increasing both TRPV1 translation (protein) [71] and transcription (RNA) [22, 72, 73] to sustain over-expression of TRPV1 in C-type nociceptors and to facilitate *de novo* expression of TRPV1 in A-delta-type nociceptors [74, 75].

Therefore long-term exposure of nociceptive terminals to inflammatory mediators such as NGF can result in long-term phenotypic changes in the repertoire of nociceptive transducing elements [76]. Extending these observations to TRPV1, experiments from several laboratories have found that NGF directs both early and long-term increases in capsaicin-mediated responses [47, 64, 77, 78]. Since it has been established that TRPV1 −/− null mice fail to develop thermal hyperalgesia following exposure to peripheral inflammation [23, 24], the

relative level of expressed TRPV1 at the site of inflammation (and probably at the spinal cord) will have a profound impact on the magnitude of inflammatory-induced pain and hyperalgesia.

Vanilloid – based therapies for the treatment of pain

Although the use of vanilloid – like creams and salves have their therapeutic origins to treat painful conditions thousands of years ago [1], their scientific and clinical uses in Western societies has only emerged since the 1800's with the isolation of the principle agent, capsaicin, from hot chili peppers. Building on the realization that the experience of pain is based on nerves (nociceptors) that respond to specific noxious stimuli that can cause tissue damage (Sherington 1906) [79], Hungarian investigators in the 1940's observed that capsaicin can both activate and inactivate sensory nerves. Following the confirmation of the 'nociceptor' hypothesis by Bessou and Perl in 1969, the existence of a 'capsaicin receptor' expressed on C-polymodal nociceptors was hypothesized. The phenomenon of nociceptor 'desensitization' due to repetitive exposure to capsaicin was finally investigated in the 1970's [80]. As shown below, it was not until the 1980's that the broader use of topical capsaicin appeared in earnest in the literature as a therapy for difficult to manage pain syndromes - especially for the treatment of post-herpetic neuralgia.

Capsaicin mediated analgesia

It has long been appreciated that initial applications of capsaicin are painful; but, paradoxically, repeated application produces a topical analgesic effect. Although a combination of mechanisms (Figure 4) including desensitization, nociceptor dysfunction, neuropeptide depletion [81, 82] and nociceptive terminal destruction [83, 84] have been proposed as critical analgesic drivers, it is most likely that the destruction of nociceptor terminals that plays the greatest role in the subsequent analgesic/therapeutic effect.

Several large double-blind, vehicle-controlled studies of patients with chronic postherpetic neuralgia (PHN) were performed to evaluate the efficacy of topically applied capsaicin 0.075% cream. The authors concluded that it was not only effective, but should be considered for the initial management of PHN [71,85]. Given the apparent initial success in the treatment of PHN, a number of other painful conditions were considered for topical capsaicin therapy. Topical capsaicin has shown promise in the treatment pain of neuropathic character including patients with complex regional pain syndrome CRPS [86]. By extension, capsaicin–based topical applications have been focused on post-surgical neuropathic pain in cancer patients using 0.075 % cream applied four times daily. Impressively, 53% vs. 17% (placebo control) of patients experienced a significantly greater pain relief while using the topical capsaicin [87]. Although an early metaanalysis that included patients suffering from diabetic neuropathy and osteoarthritis concluded that topical capsaicin improved pain when compared with a placebo, [88] the analysis includes a number of uncontrolled and/or under-powered trials, a concern that has weakened their impact over time. Moreover, if one applies a more 'rigorous' standard for clinical trials (as exists presently) on trial data prior to 2004, topical capsaicin (0.025% or 0.075 %) showed poor to moderate efficacy in the treatment of either musculoskeletal or neuropathic symptoms. [89]. Coupled with one third of these study patients experiencing adverse effects, enthusiasm for widespread use of these agents in the absence of concurrent local anesthetic pretreatment appeared to plateau and such treatments were considered for so-called, "nonresponders" rather than as a first-line treatment option.

Several aspects of topical capsaicin treatment appear to limit its overall effectiveness and application in clinical practice. The first is the requirement for repeated capsaicin application (up to 4–5 times daily) to establish and maintain an adequate degree of analgesia. Repeated use of capsaicin containing topical creams leads to the loss of epidermal nerve fibers and can

be detected as soon as three days following repeated application. In fact, after three weeks of capsaicin treatment on the volar forearm four times daily, there was an approximately 80% reduction in epidermal nerve processes. Loss of the epidermal fibers was concordant with a reduction in painful sensation to noxious and heat and mechanical stimuli [90]. Similar findings were observed when capsaicin was injected subcutaneously in volunteers [84].

Capsaicin and the skin

Although the loss of epidermal nerve fibers are also associated with localized redness and edema, there is surprisingly little damage to the keratinocytes within the epidermal layer of normal skin. Few studies have specifically reported on capsaicin-induced keratinocyte toxicity despite the reported expression of TRPV1 [91–93]. This could be the result of several factors including a lower overall level of TRPV1 expression in keratinocytes when compared with those found in dorsal root ganglia coupled with the co-expression of an inhibitory TRPV1 splice variant subunit [94]. Although there is a reduction of keratinocyte and fibroblast growth in the presence of capsaicin (0.025%), *in vitro*, at concentrations found in commercial creams [95], capsaicin may have a much greater toxic effect on keratinocytes under pathophysiologic conditions. For example, cultured cells from human squamous cell carcinoma are sensitive to capsaicin-induced apoptosis through inhibition of mitochondrial respiration [96]. In fact, certain skin disorders such as prurigo nodularis can be effectively treated with topical capsaicin [97]. Moreover, there is an increase in capsaicin receptor (TRPV1) expression in the epidermal keratinocytes and associated nerve fibers in prurigo nodularis lesions that is normalized following capsaicin treatment. This suggests that TRPV1 itself may play a critical role in both the pathology and the treatment of inflammatory disorders of the skin [98]. Therefore, it is tempting to speculate that topical capsaicin treatment would afford a selective advantage in the management of pain arising from certain cutaneous manifestations of malignant melanoma and/or squamous cell carcinoma [96, 99].

Combining capsaicin with local anesthetics

To obtain improved patient acceptance and analgesic efficacy using capsaicin based creams, therapeutic trials have progressively shifted to a combination of local anesthetic pretreatment followed by a single application of high-dose capsaicin. In a preliminary trial of ten patients suffering from intractable lower extremity pain with neuropathic features, application of capsaicin (5–10%) under regional anesthesia resulted in a wide range of post treatment pain relief [83]. Therefore, this report suggested an alternative approach, a single application period of a high dose capsaicin rather than the onerous task of repeat daily applications of low dose formulations that are associated with a high drop out rate [100].

Later, a high concentration capsaicin patch (8%) was devised and its application to the skin for a period of 1–2 hours produced longer term changes in epidermal nerve fibers that included loss of PGP-9.5 staining and reduction of heat sensitization. This illustrated that a short term application of a high concentration of capsaicin can mimic those changes previously seen under repeat application (3–5 times/day × 1 week) of lower concentration capsaicin cream [101]. Subsequently, a randomized double-blinded study, for the treatment of post-herpetic neuralgia using a one hour application of a high dose capsaicin (8%) patch was found to provide significant pain relief between study weeks 2–12 [102]. Application of the high dose capsaicin patch in this case was generally well-tolerated as it was preceded with the one hour application of 4% lidocaine jelly. Despite this, high dose capsaicin patch treatment was commonly associated with localized pain and erythema [102]. A similar study using a capsaicin (8%) patch with lidocaine pretreatment was undertaken for the treatment of painful HIV neuropathy of the lower extremities (feet) that showed modest pain relief (one third of treated patients had > 30% relief) during study weeks 2–12, without a

detectable change in the perception of warmth, cold, sharp pain or vibration sensation [103]. Interestingly, there was no apparent relationship between the duration of patch application and the degree of analgesia achieved. Adverse events included short-term site swelling and burning sensation with 44% of patients requesting oxycodone/acetaminophen following capsaicin patch placement. A small number of patients also experienced itching or coughing [103].

Is capsaicin safe?

The favorable safety profile of topically applied capsaicin relies on several complementary factors. Although capsaicin can be systemically absorbed through the skin, it does so as a function of its applied concentration and duration of exposure. When the kinetics of systemic capsaicin absorption was investigated in patients receiving a high dose capsaicin (8%) patch for pain arising from either PHN, HIV associated neuropathy (HIV-AN) or from diabetes mellitus, patch application to the trunk (PHN) directed the greatest plasma levels with the highest value observed at 17.8 ng/ml. Capsaicin is rapidly eliminated by the CYP hepatic enzyme system [104], with a population elimination half-life of 1.64 hours [105]. Significantly lower plasma concentrations were detected when the patch was applied to the feet (DN, HIV-AN). As application time was increased, (from 60 to 90 minutes) the hourly plasma concentration doubled [105].

Given this low profile of toxicity, the use of purified capsaicin solutions is being investigated for its potential to provide long-term post-operative pain relief and reduction of opioid-based analgesics following intra-operative instillation into surgical wounds [106]. Nevertheless, high doses of capsaicin inadvertently administered into the systemic circulation may produce a wide range of effects such in the pulmonary (apnea), cardiovascular (bradycardia) and thermoregulatory (hypothermia) systems [18]. The perineural infiltration of capsaicin may be another way in which to selectively target painful conditions. In fact, the idea of applying capsaicin plus a local anesthetic capable of selectively entering nociceptive fibers has been proposed and demonstrated in animal models [107, 108].

Resiniferatoxin (RTX) mediated analgesia

In comparison to capsaicin, it is surprising to find a relative paucity of preclinical and clinical trials using resiniferatoxin (RTX) as an analgesic therapy. Although RTX appears to engender many of the same benefits that were described for capsaicin, its overall structure (phorbol ester) and profile of TRPV1 activation (irreversible) likely have impacted on its translation from the bench to bedside [28, 109]. Nevertheless, a resurgence of interest in RTX has shown that intrathecal delivery in animals results in long-term analgesia – likely due to the loss of vanilloid-sensitive sensory neurons [31, 110]. Perineural application of RTX has also been shown to produce dose-dependent long-lasting analgesia [111, 112]. Importantly, analgesia was achieved in the absence of changes in proprioception or motor control [113, 114]. RTX treatment has been shown to improve nociceptive behaviors in animals with tumors and is undergoing Phase 1 and Phase 2 clinical trials to examine the safety and effectiveness of intrathecal RTX for the treatment of advanced cancer pain refractory to other treatments [113].

TRPV1 Antagonist mediated analgesia

Whereas new methodologies have been developed to apply capsaicin or RTX to appropriate target tissues, a completely different effort has been underway to develop high affinity TRPV1 antagonists with the goal of achieving analgesia by systemic administration. Ultimately, the question has become: Will blockade of TRPV1 activation reverse inflammatory pain and hyperalgesia? As previously described in detail, TRPV1 functions of

an integrator of multiple noxious stimuli serving as a cellular sensory transducer for the detection of tissue injury. It is therefore plausible that blockade of TRPV1 activation in response to the cornucopia of inflammatory mediators (protons, bradykinin, ATP, products of the lipoxygenase pathway, fatty acid metabolites, growth factors) should provide pain relief and reduce hyperalgesia. Early studies attempted to demonstrate blockade or reversal of experimental hyperalgesia in animal models but were hampered by either their lack of specificity (ruthenium red) or their low affinity (capsazepine) [115–118].

Following the isolation of TRPV1 [19], efforts to identify a high affinity antagonist were enabled with high throughput screening techniques and resulted in several promising candidates. Excitedly, an oral compound SB-705498 was shown to be effective in a human trial to reduce the area of experimental capsaicin-evoked flare when compared with placebo [119]. In addition, another orally bio-available antagonist of TRPV1 activation- AMG 517 was shown to reverse inflammation-induced pain behavior in rats. AMG 517 was later predicted to have a long half-life in humans that may be amenable to once-a-week dosing. Moreover, it was observed that blockade of TRPV1 centrally was anticipated to help provide a global analgesic effect [120]. However, during a double-blind, placebo-controlled, randomized, parallel-group, multi-center study for the management of pain following molar extraction, a test subject experienced prolonged (days) hyperthermia (> 40 °C) after taking a single 2mg dose of the high affinity TRPV1 antagonist [121]. Although this event resulted in the suspension of further Phase 1 testing of these compounds, it dramatically revealed the importance of TRPV1 in central core temperature regulation. It is uncertain whether modification of these compounds will result in a more favorable therapeutic window [122].

TRPA1: A TRP channel activated by cold, environmental irritants and cellular products of oxidative stress

Despite the central role TRPV1 plays in the transduction of multiple noxious stimuli, it has yet to be shown that TRPV1 is activated by noxious cold or high threshold mechanical stimuli. In the search for additional TRP channels capable of transducing noxious stimuli, TRPA1 (ANKTM1) was more recently isolated and characterized from sensory ganglion and found to be activated by noxious cold and irritants such as mustard oil and wasabi and certain volatile anesthetics [123–126]. Its properties of activation in response to a wide range of irritant chemicals revealed a common mechanism of activation: electrophilic-mediated covalent binding to nucleophilic cysteine [127–129].

TRPA1 is co-expressed in a subset of TRPV1 expressing nociceptors in trigeminal and DRG neurons [130] and functions to detect products of tissue injury, inflammation and oxidative stress, such as 4-Hydroxynonenal, an endogenous aldehyde that causes pain and neurogenic inflammation [128, 131] as well as prostaglandins [132]. Under conditions of inflammation/nerve injury, expression of TRPA1 is persistently increased concurrent to TRPV1 [133]. Given that TRPA1 was activated by endogenous inflammatory/oxidative stress products and implicated in mechanical hyperalgesia [134], it rapidly became a promising therapeutic target for the treatment of pain. High affinity TRPA1 antagonists are now under development and, thus far, have shown promising results in rodent models with reductions in inflammation and nerve injury-induced mechanical hypersensitivity[135, 136].

Conclusion

TRP channels TRPV1 and TRPA1 are expressed in overlapping populations of nociceptors and together function to detect noxious stimuli ranging from plant derivatives and environmental irritants to endogenous products of inflammation or oxidative stress. In the case of TRPV1, sensitization by inflammatory mediators acts to lower the thermal threshold

of activation, resulting in nociceptor activation at physiologic temperatures. Together with other members of the TRPV family plus TRPA1, these channels have the capacity to warn the body of impending tissue injury over a wide range of temperatures. Both TRPV1 and TRPA1 represent plausible therapeutic targets for novel analgesics. Since TRPV1 is expressed on polymodal nociceptors, administration of capsaicin or RTX can inactivate and/or destroy the entire nociceptive terminal. Consequently, both thermal and mechanically induced pain may be reduced. Given that many of the conditions driving tissue injury result in an increase in TRPV1 and/or TRPA1 in the nociceptors, there may be an additional therapeutic advantage. The observation that TRPV1 and TRPA1 are activated by endogenous products of inflammation established the rationale for development of high affinity antagonists. Although an 'unusual' side effect (hyperthermia) [121] has been observed in the trial of an oral TRPV1 antagonists, such agents may still be of therapeutic value if administered locally or regionally. This may be of particular importance with TRPV1, as its role in other physiologic processes from diabetes to obesity becomes clear [137, 138]. As the development of additional TRP channel agonists/antagonists advance, the goal of selectively blocking pain at its origin, at the primary afferent nociceptor, appears within reach.

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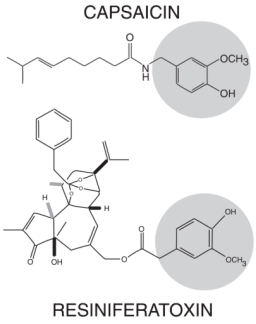

Figure 1.
Chemical structure of capsaicin (top) and resiniferatoxin (RTX) (bottom) illustrating common active moieties including –methoxy and hydroxyl groups (circled). Although both function as agonists at the TRPV1 receptor, RTX has higher potency and a characteristically slow but persistent activation.

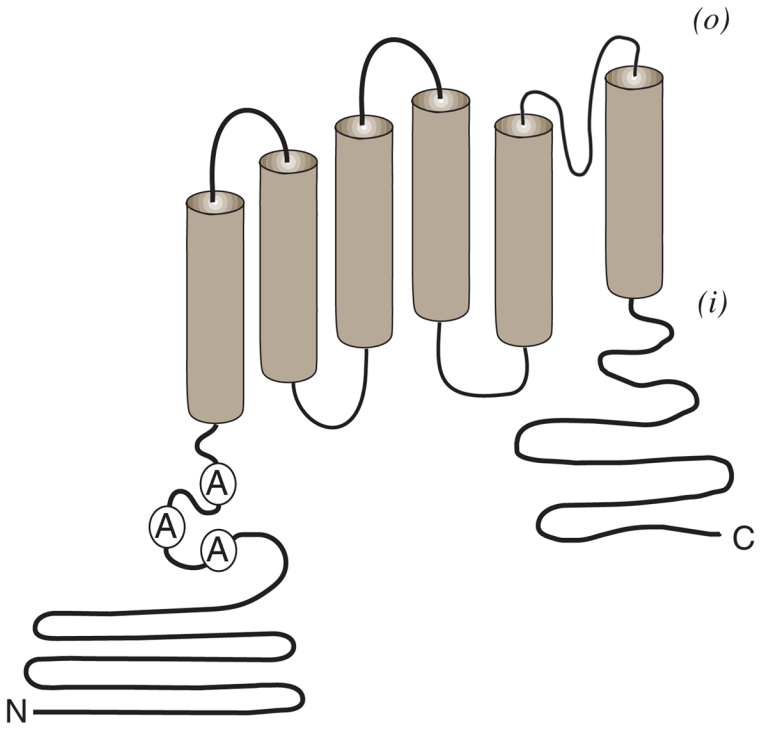

Figure 2. TRPV1 protein topology

TRPV1 is distinguished by six transmembrane spanning regions flanked by two intracellular domains (N) amino-terminal and (C) carboxyl-terminal. The N-terminal domain includes three ankyrin (A) repeat domains that may function in receptor modulation. A pore loop domain is predicted between the fifth and sixth transmembrane spanning region. It is proposed that at least four such subunits assemble to form a functional channel complex. Formation of hetermomeric channel complexes incorporating TRPV1 plus other TRPV1 splice variant and/or TRP – channel subunits have been proposed.

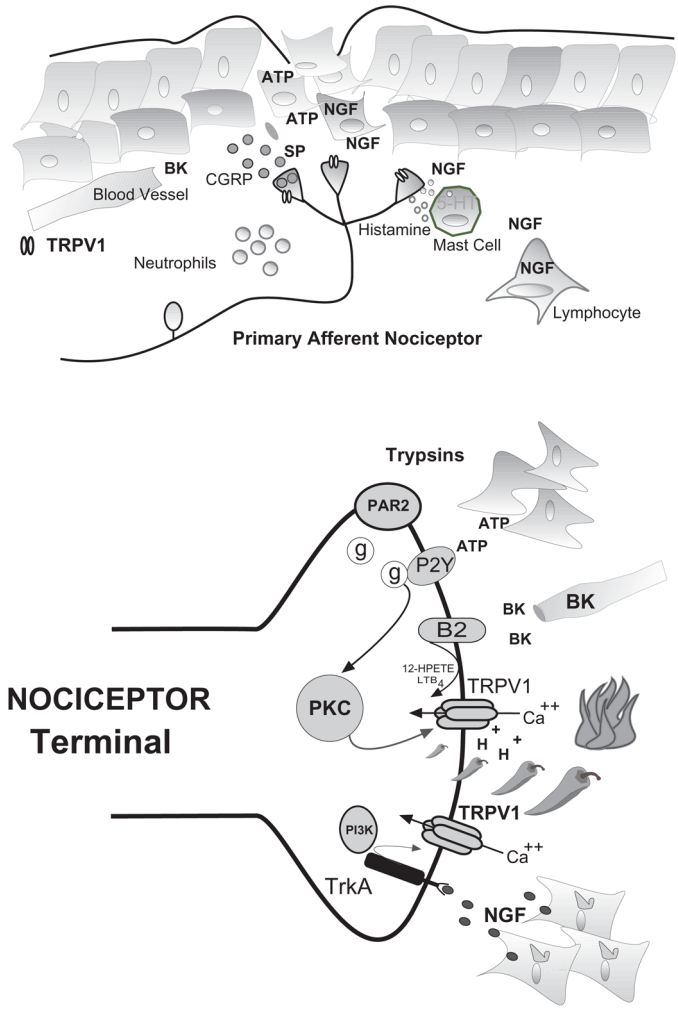

Figure 3. Mechanisms of TRPV1 mediated inflammatory hyperalgesia and pain transduction (top)

Following tissue injury, local tissue responds with increased production and accumulation of inflammatory compounds that activate/sensitize TRPV1. Nociceptive terminals derived from C- and A-delta fibers are interposed with skin fibroblasts, mast cells and the microvasculature. Following injury or inflammation terminals depolarize releasing neuropeptides substance-P (SP) and calcitonin gene related peptide (CGRP) which produces vascular leak and edema. Bradykinin (BK) cleaved from circulating kallikreins and nerve growth factor (NGF) produced by fibroblasts an infiltrating PBMs both activate and sensitize nociceptor terminals. NGF produces additional sensitization through the degranulation of mast cells containing serotonin (5-HT) and histamine. NGF and cytokines are associated with the accumulation of neutrophils and lymphocytes that participate in the maintenance of sensitization -hyperalgesia. (**Bottom**) Hypothetical nociceptor terminal expressing TRPV1 activated by capsaicin (peppers), noxious heat (fire) and extracellular protons (H^+). The resulting inward calcium current depolarizes terminal initiating action potentials that signal higher centers (not shown). Inflammatory mediators such as bradykinin (BK), ATP, trypsins, and NGF act through various secondary messenger systems to activate or sensitize TRPV1, resulting in pain and hyperalgesia.

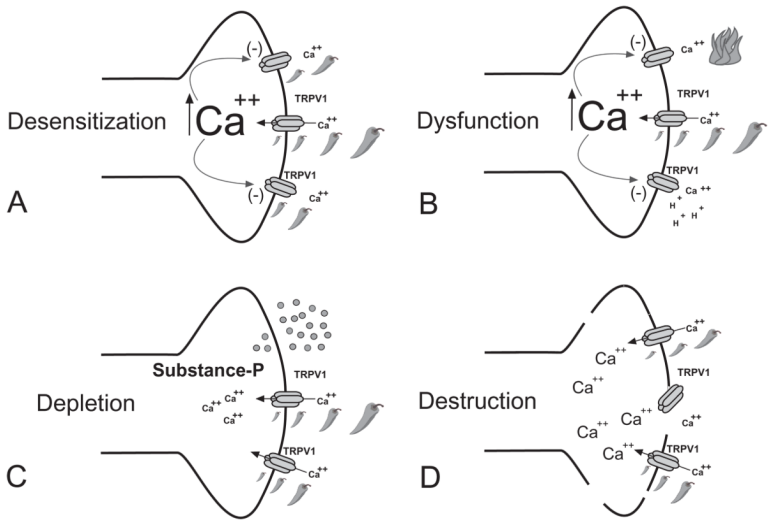

Figure 4. Mechanisms of topical capsaicin-mediated analgesia

Repeat application of capsaicin or other vanilloid – like compounds can produce a number of local effects on TRPV1 – expressing nociceptor terminals: **(A) Desensitization** is a calcium-dependent phenomenon where application of capsaicin leads to a decrease in inward current response during continued capsaicin application. When capsaicin is applied at repeated intervals, each subsequent response becomes smaller and is often referred to as tachyphylaxis. It is proposed that under these conditions, TRPV1 may also be refractory to the effect of inflammatory mediators and intracellular secondary messengers. **(B)** Repeated or prolonged application of capsaicin can produce nociceptor **dysfunction**. Under this condition, which may be secondary to an influx and/or excess of store-released calcium, other pain transducing receptor – channels may be inactivated. This could explain analgesic effects that are beyond the scope of TRPV1 function. **(C) Depletion** of neuropeptides (Substance –P, CGRP) from nociceptive terminal is evoked by capsaicin and high dose or repeat applications have been shown to deplete both central and peripheral terminals. Although the activity of Substance –P has been show to play a key role in facilitating nociceptive neurotransmission in the dorsal horn of the spinal cord, blockade of the Substance –P receptor (NK1R) has failed to show analgesia in humans. **(D) Destruction** of TRPV1-expressing nociceptive terminals has been the most reliable marker correlating the application of vanilloid –like compounds and analgesia. Although a number of mechanisms have been proposed, vanilloid – induced apoptosis appears to be the likely mechanism.

Table 1

Stimulus/Agent	Mechanism	Response	Ref
Capsaicin	Intracellular N & C terminal	Activation, Increased $Ca^{++}{}_I$ Desensitization Cell death	19, 25,26
Resiniferatoxin (RTX)	Transmembrane domain	Slow irreversible activation, Increased $Ca^{++}{}_I$ Depolarizing block Cell death	27–31
Heat	Multiple sites – C-terminal; PIP2	Activation at Temp >43 °C Increased $Ca^{++}{}_i$	19, 32–34
Acid/Base	Extracellular (H+) Intracellular (−OH)	Activation & sensitization	19,32, 35,36
Anandamide	Direct intracellular binding	Vasodilation, Activation, Sensitization	37–40
Bradykinin (BK)	12-HPETE Leukotriene B_4; PKC	Activation & Sensitization	41–43
ATP	P2Y2/PKC	Sensitization & Activation	44–46
Nerve Growth Factor (NGF)	TrkA/PI3K	Activation & Sensitization Increased expression	47, 48
Trypsins	PAR2/PKC	Sensitization	49, 50